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Fungal Mediated Conversion of Food Waste to Compost

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Abstract

Food wastage is one of the wastes released from diverse sources such as households, food processing industries, institutions, hospital, catering services and other sectors while manufacturing, managing, and processing or on consumption steps. If the food waste is managed effectively and converted to compost it would solve environmental issues. Recent studies show that composting is an effective technique for bioconversion of waste. Composting can transform food waste into hygienic, humus rich, comparatively stable products which conditions soil and enhances plant growth. Organic components of food wastes consist of vegetables, fruits, cooked food wastes and many others. This study focuses on the management of food waste and converting the waste into compost. Fruit and vegetable waste were collected from the canteen in VIT University, Vellore. The collected fruit and vegetable waste were then cleaned with distilled water. The cleaned vegetables and fruits were blended together and inoculated onto Sabouraud dextrose agar plates after serial dilution. Fungal strain thus isolated was screened for the production of different enzymes. The potent fungi isolated from fruit and vegetable waste were able to produce enzymes like amylase, protease, lipase and cellulose. These enzymes can break down complex compounds present in the fruit and vegetable waste thus accelerating the degradation process. Morphological identification was performed and the potent fungus was confirmed as Aspergillus niger. Degradation ability of food waste crude extract was analyzed using analytical technique- High performance liquid chromatography (HPLC). The potent fungus showed 95% of degradation rate post 5 days of incubation. The waste was inoculated with the isolated fungi and converted to compost by 3 months duration. Fruits and vegetable waste contribute to a large percent of food waste and hence requires a sustainable management method. Biological approach using fungus gives a higher rate of solubilization of organic compounds present in food waste compared to the conventional disposal of food waste. A significant reduction of food waste was achieved using biological methods to combat the increasing food waste crisis. These food wastes are converted to compost which can be used as a developing pathogen free soil amendment.

Keywords food waste; lipase; protease; degradation; bio compost

Introduction

The accumulation of food waste occurs all over the world due to lack of proper planning, over preparation of foods in marriage ceremonies, gatherings, hotels and restaurants, and not complying to safety policies to name a few [1]. In India about 1 lakh tones of municipal solid waste is generated per day and it is about 36.5 million tones yearly. Out of the total generated municipal waste gathered, an average of 94% is dumped on land and only 5% is composted [2]. Fruit and vegetable wastes are generated in large portions in markets and represent a source of nuisance in municipal landfills because of the sheer volume [3]. The presence of waste in the surroundings is a threat to the natural ecosystem due to their harmful effect on living beings by release of methane. The main objective behind this study was to analyse management of food waste and convert (fruit peels, vegetables) it into value added products. Various microorganisms such as bacteria and fungi have the capability to produce different enzymes like amylase, lipases, proteases etc. from these fruit wastes. The waste produced from the various food processing industries are of large quantity and to some extent they may contain value added products like compost which can be obtained from the waste products [4]. The fruit and vegetable waste mostly contains the seed, peel or the skin, pomace (pulp) and contains a variety of useful bioactive compounds. Composting occurs naturally and requires only very small energy input [5]. It is an aerobic procedure completed with the aid of microorganisms in which the organism breaks down the food waste into simple chemical free materials which could then be used in soil [6]. By redistributing nutrients and high microbial populations, compost reduces water runoff and soil erosion by means of improving rainfall penetration, which has been proven to reduce the lack of sediment, nutrients, and pesticide losses to streams by means of 75%-95% [7]. Composting in controlled environments lessens the spreading of pathogens by killing many harmful microorganisms. Composting encourages the growth of beneficial microorganisms like bacteria and fungi. These organisms can create nutritive and abundant nutrient materials called humus which helps in breaking down the organic matter [8]. They also provides minerals such as nitrogen, phosphorus and potassium to the soils and even help to retain the nutrients and keep up the balance of the soil [8].

Biodegradation has several advantages over conventional treatments of environmental cleanups as it is efficient, safer and cost effective [9]. The purpose of usage of microorganisms to degrade the waste produced can prove to be a substantial

method to deal with the growing food waste problems. The microbiome involved in the composting processes carbon as a source of energy and nitrogen to build proteins [10]. Cellulolytic fungi appear frequently in bio wastes mainly of fruits, vegetables and garden wastes [11]. The fruit waste and vegetable waste is perishable products, relatively cheap commodities and have inedible components like the peels [12]. Composting is virtually an easy and conceivable way to make a distinction inside the environmental, financial, and social troubles that the world is facing today. Adding this simple step as a part of our environmentally friendly behavior can move an extended way in reducing greenhouse emissions, regenerating the soil, revitalizing water resources, and promoting food safety into the destiny. In this study focus was given to convert the food waste (vegetable and fruit waste) into compost. Fungi was isolated from fruit and vegetable waste and screened for enzyme production such as amylase, protease, lipase and cellulase. The potential fungus was used in the process of composting of food waste, especially fruits and vegetable waste.

Materials and Methods

Chemicals

Chemicals used in the study were purchased from Hi-Media India Ltd and Sigma Aldrich. All the other reagents used in the study were of high purity and analytical grade.

Sample Collection

Fruit and vegetable waste were collected from the canteen in VIT University, Vellore. The collected fruit and vegetable waste were stored in the refrigerator at 40°C until further processing.

Isolation and Preparation of sample for analysis

The segregated fruits and vegetable pulp and peels were blended together using a mixer. The starter culture was prepared with the food waste and incubated for 10-20 days under normal environmental conditions. And at the end of the incubation period the fruit and vegetable waste mixture were completely solubilized. From these waste serial dilution was done and the diluted samples from 10^{-2} to 10^{-4} were plated into Potato Dextrose Agar plates. These plates were incubated for 3-5 days at a temperature of 37°C. And then the plates with proper isolation were selected as mother culture for further processing.

Purification of the isolated colony

The isolated colonies were purified by sub-culturing onto Potato Dextrose Agar and incubated at 37°C for 24 - 48 hours in the incubator. And the pure culture obtained was used for screening different enzyme activities.

Identification

Morphological identification was conducted to identify the isolated fungal strain from the food waste. Lactophenol cotton blue staining procedure was carried out for microscopic analysis and the colony morphology was recorded by visual identification, observation of color and also the nature of mycelial growth on the PDA plate.

Screening of enzymes

The potent fungi isolated from fruit and vegetable waste were able to produce enzymes like amylase, protease, lipase and cellulose. Enzymes are organic catalysts that permit chemical reactions to occur in living organisms at ambient situations. These enzymes can break down complex compounds present in the fruit and vegetable waste thus accelerating the degradation process.

Amylase enzyme production was checked in starch agar medium. For this the isolated fungi were grown in starch agar medium and incubated at 28°C for 3 days. After incubation the plate was flooded with 3ml of 1 % iodine and the development of clear zones around the colonies indicate amylase production.

Protease enzyme production screening was observed by plate assay using skim milk agar plate. The composition of the agar medium includes skim milk powder 28g, yeast extract 2.5g, dextrose 1 g, casein 5g, agar 15 in 1000 L with a pH of 7.0. Fungus was inoculated in skim milk agar plate and incubated at 28°C for 3 days. Clear zones around the fungal colonies after the incubation period indicate protease production [13].

Lipase activity was performed on Tributyrin agar containing glycerol oil. Isolated fungi were cultured on Tributyrin plate. and incubated at 30°C for 2- 3 days. After the incubation time the fungal colonies showing clear zones indicate lipase production [14].

Cellulase enzyme activity was checked using Carboxymethyl Cellulose Agar medium (CMC) The isolated fungal strain was inoculated on to agar plate and it was incubated at 28° C for 3 days. Congo red solution was flooded on the plate and zone of clearance around the organism was recorded [15].

Analytical analysis of food waste degradation

HPLC

High Performance Liquid Chromatography is a liquid-liquid separation technique where both the mobile and stationary phases are liquid [16]. Conformation of biodegradation of food waste was evaluated by HPLC. Degraded compounds were evaluated by using Varian HPLC with a programmable variable wavelength UV-detector, ODS2 C18 reversed phase column and binary pump. The mobile phase of 6.9 m mol/L of acetic acid in water (made to pH 4) with 35% v/v of acetonitrile at the flow rate of 1mL/min with the UV detection at 230nm was used for analysis. Injection volume of the sample was 20µl and the flow rate was 1.0 ml/min^{-1} for a run time of 20 minutes at 230nm absorption maxima. The crude food waste and post

degraded food waste supernatant was dissolved in the mobile phase for HPLC analysis.

Bio formation of Compost

For this study organic matter used was saw dust, and moisture is an important factor to support the composting process. When the compost pile is too dry, it takes time for the decaying process hence distilled water was added just enough to keep the organic matter moist [17]. To break down the waste materials (provided vegetable peel and fruit peels) by the microorganisms' oxygen is needed and to provide oxygen the compost pile was turned so that the materials at the edges moved the center in the experimental batches. This process was repeated once in 2 weeks to allow the center of the organic matter to decompose fast. The entire process took 3 months for the compost to be formed [18].

Pot study assay

The pot study assay was conducted to study the efficacy of the compost produced. For this assay Vigna radiata seeds were used. The seeds were treated with the mixture of compost (vegetable and fruit peels) and water. Seeds were grown in pots at a temperature of 30° C and left to grow for one week with 2 replicates for each treatment. One pot contained the compost soil and the other pot was filled with normal soil with no compost as the control. After the growth of the seeds the observations were made. For this the root length, shoot length and number of leaves present were noted [19].

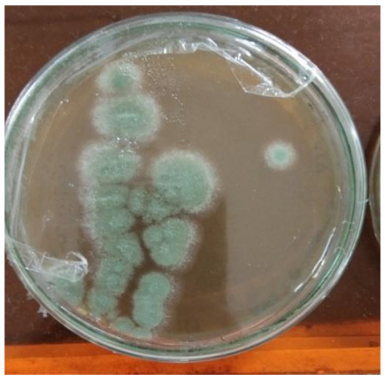

Figure 1: Potent food waste degrading isolate

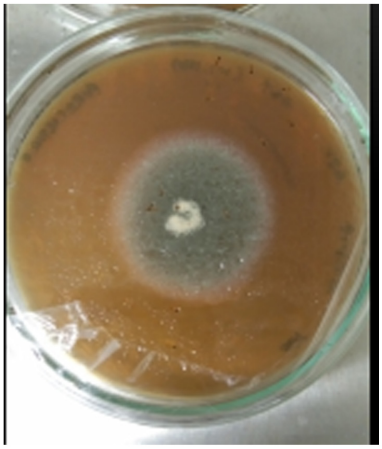

Figure 2: Isolated colony of the fungi

RESULTS AND DISCUSSION:

*Composting is the process of decaying and conversion of organic waste into manure. Due to its benefits to the gardener's compost is considered as the "black gold"[20]. The composting process requires four main ingredients: Organic matter, moisture, oxygen and microorganisms. Composting alters the nutrients in raw natural materials into a form that can be easily absorbed by the roots of plants. In the presence of a moist and oxygen abundant environment, microorganisms like bacteria and fungi fulfil the task by producing enzymes that break down or decompose the organic materials. Hence microorganisms were isolated and screened from the vegetable and fruit waste and the process of converting the food waste into compost was carried out.

Figure 3: LPCB staining of *Aspergillus niger*

Selection of mother culture plate:

The potato dextrose agar plates were observed after incubation from which 10^{-2} and 10^{-4} dilutions plates were selected and the colonies were chosen for further analysis (Figure 1 and 2).

Identification

Morphological identification was performed and the potent fungus was confirmed as *Aspergillus niger.* Lactophenol cotton blue staining was done for observing the fungal morphological characters (Figure 3).

Screening for enzyme activities

Screening of amylase activity on starch agar plate:

The species was inoculated on starch agar media and it was incubated for 3 days. When the iodine solution was poured on to the plate, we found clear zones around the colonies which indicates that amylase was produced. α -amylases are starch degrading enzyme which hydrolyses α -1,4 glycosidic bond thereby resulting in the production of short chains of dextrin compounds. In a study conducted by Francis et al. [21], *Aspergillus oryzae* showed good production of amylase enzyme. Present potent isolate, Aspergillus niger showed

Table 1: Screening of enzymes

Sl.no	Species name	Protease activity	Amylase Activity	Lipase Activity	Cellulase Activity
1.	*Aspergillus niger*	++	++	+++	++

Keywords: ++ moderate activity; +++ very good activity

Table 2. Observation of Pot study assay

Sl.no	Name of the seed	Type of soil	Shoot length (cm)	Root length(cm)	No. of leaves	Day
1.	*Vigna radiata*	With compost	14cm	3cm	2	5
2.		No compost	11cm	2.5cm	2	5
3.		With compost	17cm	4cm	2	10
4.		No compost	13cm	3cm	2	10

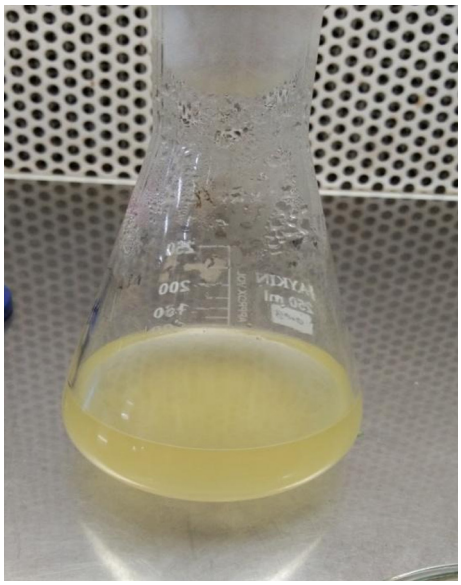

Figure 4: Culture Inoculation

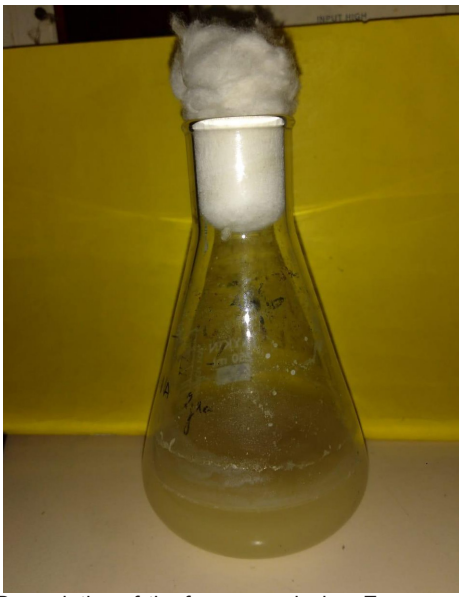

Figure 5: Degradation of the fungus producing Enzymes

better production of amylase enzyme.

Screening of protease activity on skimmed milk agar plate:

The species was inoculated on skimmed milk agar media and it was kept for incubation for 4-5 days at 28°C. After incubation zone of clearance on skimmed milk agar indicates the production of protease enzyme. Earlier reports by Souza et al., [22] reported the production of protease from Mucor pusillus and Mucor miehei isolated from the decayed milk products. Present study involves the use of *Aspergillus niger* for the extracellular production of protease enzyme. Fungal extracellular production of protease is preferred over bacteria as the fungal mass can be easily filtered during the process and also, they are recognized as generally regarded as safe (GRAS) [23].

Screening of lipase activity on tributyrin agar plate:

The species was inoculated onto tributyrin agar media and it was incubated for 3 days. The organism produces lipase as it breaks down the tributyrin it forms clear halo zones around the colonies [24]. There have been several reports stating the production of lipase from Candida rugosa, which have been used for varied biotechnological applications [25], [26]. Present study has showed prominent production of lipase using *Aspergillus niger.*

Screening of cellulase activity on carboxymethyl cellulase agar plate:

The species was inoculated onto CMC agar plate and it was incubated at 28°C for 2-5 days. Congo red solution was

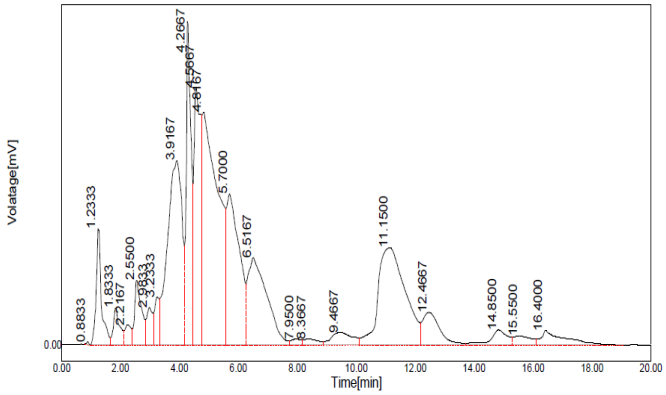

Figure 6(a): Crude food waste extract without fungal treatment

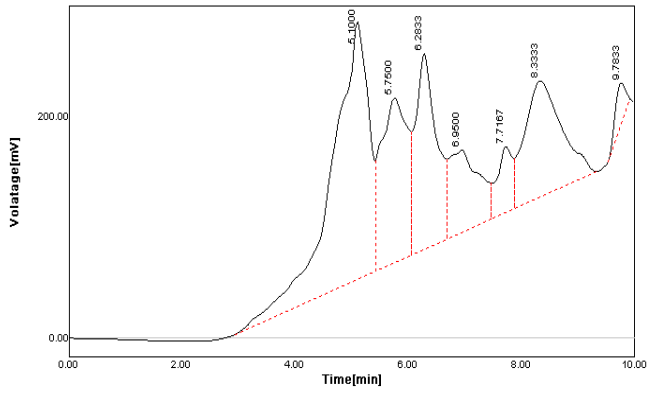

Figure 6(b): Food waste supernatant post fungal treatment

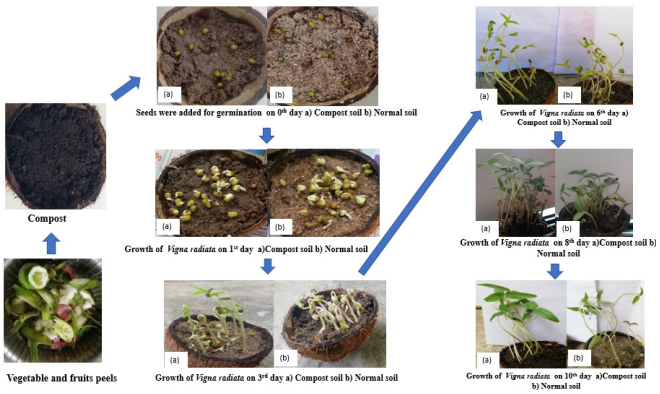

Figure 7: Flow diagram depicting the formation of compost using vegetable and fruit peels in the growth of *Vigna radiata*

flooded on the plate and we observed a zone of clearance around the species. Another study conducted by Deswal et al.,[27], showed enhanced cellulase production by a brown rot fungus, *Fomitopsis* sp. RCK2010. Potent isolate, *Aspergillus niger* showed better production of cellulase enzyme as shown in table 1.

Degradation of the food waste

The potent fungus (*Aspergillus niger*) showed a degradation rate of 95%, post 5 days of incubation. The waste was inoculated with the isolated fungi and converted to compost by a duration of 3 months (Figure 4 and 5).

Analytical technique confirmation using HPLC

Degradation of food waste by fungal isolates was confirmed using HPLC technique. Disappearance of peaks from the chromatogram of the sample was observed in comparison to the standard crude extract of food waste chromatogram (figure 6a) thereby confirming mineralization ability of fungal isolate. On the 15th day, peak disappearance was observed as seen in figure 6(b) by strain.

Pot Assay result

The assay was performed and the growth of the seeds were observed and noted down after a period of 5 days in normal temperature with equal distribution of sunlight and water. The table 2 provides their germination rate and the figures depicts the production of compost and the growth of the seeds in the compost (Figure 7).

Conclusion:

In the culture-dependent composting procedure, production of extracellular enzymes such as amylase, cellulase, lipase and protease help in the degradation of vegetables and fruits peels at a faster rate. Quantification of enzymes produced by *Aspergillus niger* helps in decomposition of kitchen refuse (fruits and vegetable peels). These extracellular enzymes help in nitrogen mineralization, phosphate and potash solubilization thereby helping in the faster growth of *Vigna radiata*. The compost was produced within a time period of 3 months, due to inoculation of the potent fungus the period of composting was reduced and the compost quality was improved. A need for proper understanding is still lacking about the effect of the microorganisms on the compost production and also comparing expensive downstream processing of the composting are the main problems faced towards a low-cost compost production. Annually huge quantities of food waste are produced worldwide There are several advantages and limitations present for the conversion of these food waste into value added products, and for converting these-waste there is a proper lack of technology for the efficient conversion.

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